Improving honey bee health and food security through breeding and IPM control of parasitic mites

Michael Simone-Finstrom[1], PI
Michael.Simonefinstrom@usda.gov
225 421-9155

Arian Avalos[1], Co-PI
Arian.Avalos@usda.gov
225-276-3686

Frank Rinkevich[1], Co-PI
Frank.Rinkevich@usda.gov
225-276-3998

Elizabeth Walsh[1], Co-PI
Elizabeth.M.Walsh@usda.gov
225-767-9280

Vincent Ricigliano[1], Co-PI
vincent.ricigliano@usda.gov
225-236-7694

Steve Cook[2], Co-PI
steven.cook@usda.gov
301-504-0416

Julia Fine[3], Co-PI
julia.fine@usda.gov
530 754-1383

Gloria DeGrandi-Hoffman[4], Co-PI
gloria.hoffman@usda.gov
520-647-9187

Mark Carroll[4], Co-PI
mark.carroll@usda.gov
(520) 647-9322

Perot Saelao[5], Co-PI
perot.saelao@usda.gov
830-223-2788

[1]USDA-ARS Honey Bee Breeding, Genetics, and Physiology Research Lab (HBBGPRL), 1157 Ben Hur Rd., Baton Rouge, LA 70820

[2]USDA-ARS Bee Research Laboratory (BRL), 10300 Baltimore Ave, BLDG. 306, RM. 315, BARC-EAST, Beltsville, MD 20705

[3]USDA-ARS Invasive Species and Pollinator Health (ISPH), 800 Buchanan St, Albany, CA 94710

[4]USDA-ARS Carl Hayden Bee Research Center (CHBRC), 2000 East Allen Rd, Tucson, AZ 85719

[5]USDA-ARS Veterinary Pest Genetics Research Unit (VPGRU), 2700 Fredericksburg Rd, Kerrville, TX 78028

2. Executive Summary: Honey bees contribute billions of dollars of agricultural productivity through their extensive pollination services and produce millions of pounds of honey annually. In light of this crucial importance, knowledge that colony losses have approached 40% annually is concerning. While many factors affect honey bee colony health and performance, the parasitic mite, *Varroa destructor*, has an outsized impact due to their damaging feeding behavior, ability to transmit viruses, and their ubiquity throughout North America. Beekeepers have long used miticides to control *Varroa*, but the recent development of resistance to amitraz, the most widely used miticide in the U.S. and Canada, strongly indicates new approaches are urgently needed to effectively control *Varroa*. The goals of this Areawide proposal will apply *Varroa* management strategies that have been developed at honey bee labs across ARS. We will use a tech transfer approach to train beekeepers how to quantify suppressed mite reproduction (SMR), an estimate of *Varroa* Sensitive Hygienic (VSH) trait. This will help them identify breeder queens within their own operation promoting the dissemination of these genetics on a wider scale (Objective 1). We will use genetic analyses to develop a marker assisted selection (MAS) program to facilitate breeding of the SMR trait. This will allow for rapid selection of breeder queens (Objective 2). Evaluating amitraz resistance genes will be offered so beekeepers can obtain information on the resistance status of their *Varroa* and make data-driven treatment decisions. Efficacy of treatment regimens will include evaluating novel miticides across a broad geographic range to develop a field decision tool (Objective 3). Finally, an economic analysis will be conducted to demonstrate cost savings and increased revenues possible with incorporation of integrated *Varroa* management strategies to further incentivize implementation (Objective 4). This large-scale tech transfer event will happen over the next 5 years with ARS labs that conduct honey bee research in cooperation with our commercial beekeeping stakeholders across the country. Research has shown promise for many new effective *Varroa* management techniques and this project seeks to leverage and communicate those findings to deliver both immediate and long-term solutions to *Varroa* in the beekeeping industry.

Keywords: Honey bee, *Varroa destructor*, selective breeding, marker assisted selection, miticide resistance, beekeeping economics

3. Problem Statement and Justification: Honey bees (*Apis mellifera*) are integral to agriculture and food security for their pollination services which are valued at over $15B annually[1] and the production of 138M lbs of honey annually valued at nearly $350M[2]. However annual honey bee colony losses across the U.S. have been nearly at 40% over the past two decades, making our reliance on them uncertain[3]. Though beekeepers have been able to "keep up" by making more splits form starting colonies, this leads to overall smaller, less productive colonies and lower survivorship over winter. This contributes to the major decline in overall honey production in the U.S. over the last several decades and the unsustainable losses seen recently[4]. Presently, much of the resulting economic burden from loss is borne by the Emergency Livestock Assistance Program (ELAP) with annual payments to beekeepers sometimes exceeding $50M annually. The single most significant factor contributing to these losses is the parasitic mite, *Varroa destructor*, which destructively feeds on bees[5,6], weakening them and exposing them to a variety of deadly viruses[3,7]. By addressing key factors like *Varroa* and developing regionally aware control strategies, our strategy aims to systematically increase industry gains while reducing operational and systemic (ELAP) losses.

The present strategy for mitigating the impacts of *Varroa* is primarily through chemical control (miticides) which has led to over-reliance and overuse within the beekeeping industry[8,9]. This is particularly a problem for commercial beekeepers, whose responsibility as stewards of most pollination service and honey production colonies is too sensitive for risking untested or potentially costly alternatives or time-intensive cultural pest controls. This is not sustainable and resistance to chemical controls, a common phenomenon in other systems, is on the rise[10]. This creates a need for beekeepers to have access to resistance information within their own operations to make effective treatment decisions. In addition, novel treatments and control strategies are needed to overcome the risk of increasingly widespread resistance and resistance to other miticides. Novel molecules[11], formulations[12], application strategies and cultural control methods are available, but data is lacking on their efficacy in beekeeping operations.

With the global increase in amitraz resistance, it has become clear that breeding efforts need to be at the forefront of *Varroa* control and must be the real goal to increase the sustainability of beekeeping and promote food security. Chemical treatments are still needed as a stop gap, but breeding of resistant honey bee populations is the ultimate sustainable control mechanism. Breeding efforts have become more widespread but need a concerted effort for deployment to stakeholders. HBBGPRL and aligned collaborators have been leaders of many of these efforts, developing proven identification and phentoyping methods for *Varroa* resistance[13] in honey bees as well as establishing multiple resistant stocks[14,15]. Specifically, HBBGPRL has developed Pol-line[15], a unique population exhibiting *Varroa* specific hygienic behavior, a trait proven to eliminate nearly all *Varroa* in hives[16]. However, the population has limited distribution among beekeepers and their performance has only been evaluated in a few geographic regions. HBBGPRL has also led efforts in the use of genetic tools for honey bee breeding[17,18]. Global efforts to advance breeding via genomic tools have also made significant progress, but we need to leverage those efforts and make them practical for stakeholder buy in.

This project seeks to develop an integrated control management strategy for *Varroa*. Participating co-PIs and collaborators are at the forefront of honey bee research. Funding, if obtained, would help integrate their efforts into a deliverable program and practical management practices for the beekeeping industry. The unique resources at the HBBGPRL and collaborating institutions make the proposed work reasonable and the goal of sustainable *Varroa* control and thus a more secure U.S. beekeeping industry allowing reliable pollination services a reality.

4. Project Objectives:

Objective 1: Increase implementation of mite resistant bees in beekeeping. Selection on suppressed mite reproduction (SMR) phenotyping is a proven breeding strategy for lowering the effect of *Varroa* mites on honey bee colonies and mitigating much of their impact. A major limitation to the broad availability of *Varroa* mite resistant stock has been a lack of a comprehensive tech transfer strategy. We aim to establish a program that works to directly export and integrate knowledge the methods towards selecting for mite resistance to honey bee breeding operations in order to establish their widespread use.

Objective 2: Advance the utility of genomic tools to select for resilient bees. Honey bee queen producers and bee breeders have not yet implemented marker assisted selection (MAS) as is common in other livestock species. Engagement and knowledge transfer of this process and its value to increase efficiency of breeding efforts is key to stakeholder adoption of these techniques. HBBGPRL is currently collaborating with multiple groups to finalize the development of a genotyping panel for the SMR trait, providing an opportunity to establish the use of MAS in honey bee breeding programs. We aim to develop a training program towards the integration of MAS for the SMR trait and use this as a launching point for more advanced, multi-trait selection approaches, like genomic selection, for population-specific breeding efforts.

Objective 3: Create field decision tools for monitoring and managing miticide resistance in *Varroa*.

The widespread use and reliance on amitraz-based products has led to increased prevalence of amitraz resistance throughout the U.S. This objective seeks to make amitraz resistance genotyping and resistance testing more accessible and efficient so beekeepers can make timely and accurate *Varroa* treatment decisions within their own apiaries. We will also evaluate the efficacy of novel control materials and formulations, the use of miticide rotations or co-applications with various modes of action, the implementation of mechanical and cultural controls to enhance miticide applications, and the use of antiviral treatments to reduce disease transmission by *Varroa*.

Objective 4: Evaluate the economics of *Varroa* control strategies and the additive effects for beekeeping operations.

Varroa control is costly, but typically the cost of no *Varroa* control is greater because it results in colony loss. However, little is known about the net costs from partially adequate *Varroa* control, which allows honey bee colonies to survive but likely leads to lost revenue due to reduced colony productivity and colony strength, and thus pollination services. This knowledge gap can lead to poor decisions regarding the value of breeding for mite resistant bees, miticide usage, and additional management methods by beekeepers. In order to fully understand the economic outcomes of parasite management decisions, we will develop models to holistically assess cost from lost products due to inadequate *Varroa* control along with benefits of different pest control approaches to determine the best cost-benefit scenarios and share them with beekeepers.

5. Plan of Work

A. Operations: The proposed aims to establish a multi-institutional program to incite a paradigm shift towards more effective and sustainable control of the *Varroa* mite. Our approach centers around development and deployment of an integrated pest management (IPM) program that capitalizes on complementary research from across four USDA-ARS laboratories and close collaboration with stakeholders across the U.S. The HBBGPRL will act as lead Unit in the project using VSH stock developed at the HBBGPRL to 1) increase the prevalence of *Varroa* resistant honey bees in U.S. beekeeping, 2) advance honey bee breeding through integration of genomic tools, 3) establish a holistic *Varroa* IPM plan preventing mite damage and development of miticide resistance, 4) assess economic impacts for stakeholders based on mite control strategies used. Each objective addresses a critical need for effective *Varroa* IPM and outlines contributions from stakeholders and collaborator-specific efforts.

The first objective of our strategy is to increase identification and development of VSH honey bee stock in commercial beekeeping. Past and continuing work at HBBGPRL has demonstrated the efficacy of selection based on SMR phenotyping resulting in VSH honey bee populations such as Pol-Line. Presently, a major limitation has been adoption of these phenotyping methods by stakeholders. A key component of this barrier is the lack of a comprehensive educational process to transmit the methods for SMR trait selection to stakeholders (honey bee breeders) interested in establishing resistant stock within their own operations. This has led to limited availability of mite-resistant stock during a period where effects from *Varroa* mite parasitism are driving clear, consistent losses across honey bee affiliated industries.

The proposed work directly addresses the barrier to education through development and deployment of a comprehensive engagement and outreach program. Initial efforts will compile and disseminate information on VSH trait identification through best-practice guidelines for the SMR phenotyping approach and how to integrate it within breeding operations. This will be achieved in direct collaboration with stakeholders with on-site regional training targeting beekeepers nationwide, coordinated by Dr. Julia Fine (ISPH), Drs. Avalos, Rinkevich, and Simone-Finstrom (HBBGPRL) and Dr. Robyn Underwood and Kate Anton of Penn State University, State College, PA. Regional workshops will present critical information and provide hands on training to measure SMR in colonies, logistics of selective processes to establish the trait in populations, and how to monitor trait performance. HBBGPRL will provide follow-up consultation and compile outcomes from the collaborative group to continually improve best-practices guidelines for selection using the SMR phenotype, breeding strategies, and maintenance.

To enhance the effectiveness of our tech transfer and engagement program, additional data on the performance of VSH stock is needed. Specifically, information is needed on maintenance of VSH stock across a variety of ecological regions. We propose to address this need through longitudinal monitoring of established VSH honey bees (Pol-Line) across a U.S.-wide range of monitoring sites. Specific sites will include HBBGPRL, ISPH, and also monitoring by Dr. Gloria DeGrandi-Hoffman (CHBRC), and Dr. Steven Cook (BRL). Target data includes parameters of colony health, growth, and production collected over project years to develop region-aware best-practice guidelines for the use of VSH stock.

The second objective aims to complement phenotyping efforts through the use of genetic-informed approaches like MAS and genomic selection. Though widely available in other agricultural animal and crop systems, the integration of MAS and genomic selection in honey bee breeding has not been readily adopted. The principal barrier to this has been a lack of expertise and familiarity with novel advances. HBBGPRL has already established a collaboration with Breeding Insight to facilitate genetic screening and interpretation tools for stakeholders. Furthermore, present advances in our understanding of several traits of interest in honey bees including VSH is quite advanced. HBBGPRL is at the forefront of VSH trait characterization, collaborating in two independent projects aiming to develop genotyping panels for VSH. The Unit also is involved in multiple projects developing U.S.-wide reference panels with the aim of applying the information towards multi-trait selection via genomic selection.

We propose to leverage established collaborations to deploy a genotyping pipeline for MAS targeting the VSH trait and to introduce stakeholders to the use and application of genetic tools. Once established we will introduce the use of genomic selection. Alongside interested stakeholders, we will establish and train participants on the use, application, and interpretation of VSH MAS. Development of the MAS pipeline and stakeholder training will be conducted in years 1-3 of the proposed work. Once stakeholders are familiar with MAS, we will introduce genomic selection methods in years 4-5. This initiative will be supported through ongoing research on the application of genomic selection in honey bees led by HBBGPRL in collaboration with Dr. Garett Slater at Texas A&M University, and Dr. Perot Saelao (VPGRU). By presenting genomic selection from an initial introduction to MAS, the HBBGPRL aims to demonstrate how minor modifications in breeding practices can yield significant improvements in trait values, thereby advancing the efficiency and effectiveness of breeding in the beekeeping industry.

Breeding is the ultimate goal for sustainable control of *Varroa*, however chemical controls are still needed as a stop gap control method. For over 25 years, amitraz has been the almost exclusive miticide control of choice in commercial beekeeping because of its efficacy against *Varroa* and minimal impacts to honey bee colonies[9]. However, increasing global levels of amitraz resistance and related increases in treatment failure threaten the sustainability of beekeeping in the U.S. and abroad[10]. It is critically important to mitigate the further development of amitraz resistance in *Varroa* populations. Lacking is information on its spread and data-based assessments of alternative chemical, mechanical, and cultural control strategies that can limit miticide resistance in *Varroa*. In addition to damage from feeding, *Varroa* are known vectors of deadly honey viruses. Though antiviral treatments show success, no commercial application is available. The HBBGPRL has led the development of a novel feed-based antiviral therapeutic. We will use these therapeutics in a migratory beekeeping operation to additionally evaluate how reducing viral load transmitted by *Varroa* mediates their impact on honey bee colonies and economically impacts beekeeping operations.

The proposed aims to establish a more accessible and rapid testing of amitraz resistance in *Varroa* populations (Objective 3). The HBBGPRL will establish an online portal to inform stakeholders on resistance testing and coordinate to provide self-administering testing kits. Information on the portal will include testing protocols, datasheets, and a video tutorial. An automated data processing section will also be established to allow for instantaneous evaluation of results and inform treatment decisions. Amitraz resistance is known to be genetically determined by a mutation of octopamine receptor subunits[19,20]. Having a genetic target allows for the rapid evaluation of amitraz resistance alleles. In collaboration with the Bee Disease Diagnosis Service at Beltsville, we aim to validate and establish protocols for such a service.

The fourth objective emphasizes conveying economic data on *Varroa* management. Specifically, economic assessments will focus on cost / benefit analyses of current control strategies on hive products (e.g., queens, nucs, packages, honey, pollination services, and more). While the economics surrounding no *Varroa* control directly are well understood, the economics of utilizing various *Varroa* control methods discretely and simultaneously are only beginning to be evaluated[21]. Since American beekeepers do not currently know the potential economic impact of various *Varroa* mitigation strategies, this objective aims to both collect this data and inform BMPs considering these results. This information will be disseminated to stakeholders and stakeholder-serving entities towards enhancing industry-wide outcomes.

B. Assessment: A successful Areawide program will show reduced honey bee colony losses through increased use of *Varroa*-resistant stocks and effective miticide applications resulting in a net positive economic impact on beekeeping operations. The annual change in the proportion of colonies in a beekeeping operation that are identified as breeding stock will be measured in participant operations by CO-PIs and collaborators in Objective 1. Participants in the regional workshops on SMR phenotyping will be surveyed by instructing collaborators to determine understanding and improve the information and presentation methods. Adoption of genomic tools for MAS of *Varroa* resistance and detection of amitraz resistance alleles will be tracked by the number of samples processed and market research surveys to measure the likelihood and willingness to adopt genomic tools. Reductions in miticide applications due to increased use of resistant honey bee stock will be tracked through assessment of miticides in wax coupled with surveys on miticide use patterns, which are parts of an ongoing USDA-SCRI grant with Dr. Rinkevich. Annual colony loss surveys are conducted by Dr. Geoff Williams at Auburn University and the USDA National Agricultural Statistics survey in such a way that changes in colony losses at the start of the program can be compared colony losses at the end. This will allow us to track the changes in colony losses during project years. The number of colonies lost and compensation paid through ELAP will be tracked to assess economic impact of Areawide initiative. A tailored survey at the end of the program will demonstrate the number of beekeepers using resistant stocks in their operations at the end of the program. Data and information gained from economic studies in Objective 4 will demonstrate progress in improved breeding for *Varroa*-resistance or areas where additional progress is needed, allowing the assessment of previous efforts (Objective 1-3) to incorporate improved breeding practices into beekeepers' operations.

C. Research: ***Improve knowledge of management of mite resistant bees across climates.*** The success of U.S.-wide deployment of VSH stock is predicated on accounting for significant geographic variation across climatic zones. Additionally, colony density and areas with a combination of mite resistant and mite susceptible stocks need to be investigated with particular respect to understanding impacts of mite drifting among colonies[22,23]. This is particularly salient for commercial operations as their large-scale deployment of colonies usually necessitates physical nearness. Mite drift may be further exacerbated by increasing temperatures which may extend the period during which it occurs[24]. Cold storage of colonies has arisen as a method for mitigating this impact[25,26], however this approach is nascent. Here, CHBRC will expand current research to inform management strategies for Pol-line and SMR-selected stocks.

MAS validation and population specific genomic selection panel. Effective integration of MAS in honey bee breeding programs is predicated on availability and relevance to stakeholder populations. Efforts at HBBGPRL are finalizing two VSH trait marker panels. One U.S.-based effort is stem from a collaboration between HBBGPRL with University of Missouri, and a separate, international collaboration with AristaBee surveys European populations. Both will result in a comprehensive pool of markers which here we propose to assess and validate with colonies from participating bee breeders in this proposed work. The expanded panel derived from the union of these data sets be tested in drone samples from stakeholder colonies with SMR values, mite population growth estimates, and standard colony traits (supersedure, production, viral tolerance) over the first project year. The overall goal will be to arrive at a condensed set of markers for a U.S.-specific selection panel. Data from this effort will also be directly used in later project goals to develop a reference set for genomic selection.

Informing effective miticide use as a stop gap for Varroa control. Key data is needed to make resistance testing of *Varroa* populations more approachable to stakeholders to allow for timely treatment decisions. An important component is field deployment of the testing process. Here we will examine Amiflex, a new formulation of amitraz marketed as a rapid treatment which has the potential to reduce the resistance testing process by 2 hours[10]. This will be paired with improving the genotype validation process, which presently uses individual *Varroa*, a costly and time-consuming procedure. Specifically, we will modify our current genotyping method[10] to be applied to pooled samples of *Varroa* and analyzed in the field using portable devices (e.g. Solas 8). Together this would make two independent tests of amitraz resistance available for beekeepers and apiary inspectors to readily apply as part of their standard inspection routines.

Research is urgently needed for novel miticidal molecules, formulations, and application strategies. Molecules like fenazaquin and fenproximate have been identified and tested on a small scal[11]e while formulations like extended-release oxalic acid are available and nascent in their use. Also needed are assessments of possible effective application strategies like co-application or pairing products with different modes of action or rates of efficacy, in addition to working with participating stakeholders in their own operations to assess new miticide candidates and antiviral therapeutics in mite resistance bees with and without *Varroa* presence. The lack of large-scale testing has limited adoption of many of these control methods. Here we will capitalize on the national network of our project team to gather critical data across a broad geographic and climactic gradient to assure regionally informed BMP[12,27].

D. Outreach and Technology Transfer: All members of this proposal excellent relations with our stakeholders often interacting at their bee yards and in meetings. We will directly train cooperating beekeepers using their own colonies as outlined in Objectives 1-3 via the development of a Tech Transfer Team located at HBBGPRL. Regional training opportunities in CA, LA, and PA will train attendees on evaluating and selecting for VSH using prepared resources and facilities. Workshops to educate beekeepers on selection for VSH will be offered at the annual meetings of the American Honey Producers Association (AHPA) and American Beekeeping Federation (ABF). Field demonstration of miticide applications and *Varroa* inspections will be held at annual meetings of the Eastern Apicultural Society (EAS) and Heartland Apicultural Society (HAS). Amitraz resistance diagnostic testing will be available to beekeepers through the Bee Disease Diagnosis Service at Beltsville. Progress on our objectives will be presented in person and virtually at regional and national beekeeper meetings, published in trade and open access scientific journals, and used for Best Management Practices Guides.

6. Project Team:

While Dr. Michael Simone-Finstrom is the lead project investigator and will be responsible for coordination and oversight of all project aspects, Drs. Arian Avalos and Frank Rinkevich are overall co-leads and will participate in all major aspects of the project and co-supervise staff hired on to support the work. See Table 1 for more information.

An advisory board will be established, consisting of national and regional industry partners, including leadership from the major honey bee stakeholder groups—the American Beekeeping Federation and the American Honey Producers Association, the major group representing queen production—California Bee Breeders Association, and the major industry non-profit supporting applied honey bee research—Project Apis m. This will help ensure that the goals and outputs remain tied directly to industry needs and allow us to adjust materials, information, and training as needed to increase effectiveness and impact.

Table 1. Project team details.

Location	**Person**	**Role**	**Objective(s)**	**% Time**
USDA-ARS, SEA Baton Rouge, LA	Michael Simone-Finstrom	PI	Overall, Lead Obj. 1; Obj. 2 and 4; Supervise Tech Transfer Team	15
	Arian Avalos	Co-PI	Lead Obj. 2; co-lead Obj. 1, Obj. 4	10
	Frank Rinkevich	Co-PI	Lead Obj. 3; co-lead Obj. 1; Obj. 4	10
	Elizabeth Walsh	Co-PI	Lead Obj. 4	2
	Vincent Ricigliano	Co-PI	Obj. 3, lead antiviral testing; Obj. 1	2
USDA-ARS, NEA Beltsville, MD	Steven Cook	Co-PI	Obj. 3, lead novel miticide; coordinate analysis of *Varroa* samples sent to Diagnostic Lab; Obj. 1 test location for management	5
USDA-ARS, PWA Davis, CA	Julia Fine	Co-PI	Obj. 1 and 2; test location for management, coordination with CA queen producers and training	5
USDA-ARS, PWA Tucson, AZ	Gloria DeGrandi-Hoffman	Co-PI	Obj 1 and 4; lead on mite drift and cold storage	5
	Mark Carroll	Co-PI	Obj 1, mite drift and test location for management	5
USDA-ARS, PA Kerrville, TX	Perot Saelao	Co-PI	Obj. 2	1
UC Davis	Brittney Goodrich	Collaborator	Obj. 4	1
Texas A&M	Garett Slater	Collaborator	Obj. 2	1
Penn State University	Kate Anton	Collaborator	Obj. 1, assist in coordination of training in NE region	1
	Robyn Underwood	Collaborator	Obj. 1, assist in coordination of training in NE region	1

7. Project Coordination, Reporting, and Assessment:

Project Coordination: All components of the project will be coordinated through HBBGPRL based on the timeline presented below (Table 2). Overall, M. Simone-Finstrom will be responsible for reporting of the full project and Obj. 1, A. Avalos for Obj. 2, F. Rinkevich for Obj. 3 and E. Walsh for Obj. 4. The majority of budgetary funds will be used to create and staff a Tech Transfer Team (one postdoc, 2 GS-7 term technicians, and seasonal employees), overseen by M. Simone-Finstrom. By creating this team there will be a crew that entirely focuses just on connecting with our stakeholder partners, sampling and transferring knowledge directly in their operations to allow them to make real-time decisions.

Any potentially needed corrections in experimental designs, technology transfer strategies and coordination of duties will be discussed at quarterly PI progress meetings. PIs involved in a particular objective will each review the development of outreach, teaching and technology transfer materials for clarity and content, along with a review by our advisory board. Through these meetings and discussions, along with results of participant and stakeholder surveys, we will be able to clearly identify areas that need improvement, coordinate plan of action and implement changes immediately through these team efforts.

Table 2. Project timeline presented quarterly for years 1-5. Investigator or collaborator locations are indicated by the number in each section. 1: HBBGPRL, Baton Rouge, LA; 2: BRL, Beltsville, MD; 3: ISPH, Davis, CA; 4: CHBRL, Tucson, AZ; 5: VPGR, Kerrville, TX; 6: Penn State University

	Y1				Y2				Y3				Y4				Y5			
Objective 1	W	Sp	Sum	F	W	Sp	Sum	F	W	Sp	Sum	F	W	Sp	Sum	F	W	Sp	Sum	F
Establish outreach material and BMP	1	1	1	1	1				1				1				1			
Training workshops			1,6	1,3	1		1,6	1,3	1		1,6	1,3			1,6	1,3	1		1,6	1,3
Inform mite-resistant colony managment		1,2,3,4	1,2,3,4	1,2,3,4	1,2,3,4	1,2,3,4	1,2,3,4	1,2,3,4	1,2,3,4	1,2,3,4	1,2,3,4	1,2,3,4	1,2,3,4	4	4	4	4	4	4	4
Objective 2																				
MAS panel validation	1,5	1,5	1,5	1,5																
MAS integration				1	1	1		1	1	1		1	1	1		1	1	1		
GS reference development and integration														1,3,5	1,3,5	1,3,5	1,3,5	1,3,5	1,3,5	1,3,5
Objective 3																				
Refine amitraz resistance testing		1,2	1,2	1,2			1,2	1,2			1,2	1,2			1,2	1,2			1,2	1,2
Combined control method assays		1	1	1		1	1	1		1	1	1								
Novel control method testing			1,2	1,2			1,2	1,2			1,2	1,2			1,2	1,2			1,2	1,2
Antiviral testing														1	1	1	1	1	1	1
BMP guidelines developed and refined					1,2				1,2				1,2				1,2			
Training workshops							1,2	1,2			1,2	1,2			1,2	1,2			1,2	1,2
Objective 4																				
Surveys and economic analyses				1,4	1,4			1,4	1,4			1,4	1,4			1,4	1,4			1,4

Reporting: Quarterly virtual meetings will be held to coordinate work for each objective, discuss progress, and address issues. Annual reports will be presented at the virtual PI meeting along with written progress reports.

Progress on our objectives will be presented in person and virtually at regional and national beekeeper meetings such as AHPA, ABF, American Bee Research Conference (ABRC), EAS, HAS, At Home Beekeeping from Alabama Extension Services, and many state organizations. Research results of all objectives will be published in peer-reviewed journals such as *Apidologie*, *Journal of Economic Entomology*, and *Journal of Apicultural Research* as well as in trade publications such as *American Beekeeping Journal* and *Bee Culture*. Economic results will also be incorporated into a reference resource for beekeepers attending workshops and field demonstrations. Best Management Practices manuals and webpages for mite resistance and mite treatment will also include economic findings (Obj. 4) and be created in collaboration with state extension agents and cooperators such as the Honey Bee Health Coalition and distributed openly through these connections and our strong contacts with the Apiary Inspectors of America. MAS/genomic selection will be made into a readily available commercial service with help of Breeding Insights, with whom HBBGPRL has an established collaborative relationship.

Assessment

Table 3. Knowledge gained by stakeholders and economic, environmental and societal impacts.

Measurement	Method	Defined Successful Impacts	PIs
Proportion of breeder queens within a beekeeping operation	Colony inspections and database reporting	An increase from the initial low frequency to more than 75% of colonies making grade as breeders	AA, MSF, FDR
Impact and scope of workshops for identifying SMR	Participant Surveys	More than 80% of participants implementing SMR measurements in their breeding operations	MSF, AA, FDR, JF
Use of genomic tests for MAS for SMR	Samples processed	Annual increases in the number of samples processed and expansion of services	AA, MSF, PS
Adoption of genomic tools for breeding	Market research survey	More than 70% of respondents indicating somewhat likely or very likely to use these services	AA, MSF
Detection of amitraz resistance allele frequencies	Samples processed, participant surveys	Annual increases in the number of samples processed; reduced frequency of resistant alleles; changes in IPM strategies based on results	FDR, SC
Impact of IPM strategies	Outreach materials, trade journal articles, participant surveys	Reduction of colony losses due to *Varroa* supporting honey production and pollination services; increased use combination of mite control strategies	MSF, AA, FDR, EW
Miticide use	Wax residue analysis Survey Economic analysis	Reduction in miticides present in wax; reduced reliance on a single control strategy; reduction in treatment cost	FDR, SC
Adoption and widespread advocacy for full IPM strategy in commercial operations	Annual meeting with stakeholder advisory board	Active participation in key stakeholder meetings initiated by stakeholder groups; inclusion in programming and resources through stakeholder outlets	MSF, AA, FDR, EW, VR, GDH, JF, SC

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